Novel variation in *ENG* gene causing Hereditary Hemorrhagic Telangiectasia

To detect exon mutations in the *ENG* gene, we designed 32 primer pairs using *Primer3* and *PrimerBLAST* online software. Primers were designed for the 15 exons of the *ENG* gene (NM_000118.3) and were manufactured by BioresearchTechnologies.

Genomic DNA of a Peruvian family diagnosed with Hereditary Hemorrhagic Telangiectasia was analyzed by Sanger sequencing for the 15 exons of the *ENG* gene. The family is conformed by 4 affected individuals (IV-2, V-1, V-2, VI-1) and 1 unaffected member (VI-2) (Figure 1). We performed Sanger sequencing of purified PCR products using GeneJET PCR Purification Kit (Thermo Fisher Scientific, Boston, MA, USA), BigDye Terminator v3.1 Cycle Sequencing Kit and the ABIPRISM 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The generated sequences were analyzed using FinchTV software compared to GRCh37.p13 assembly.

We performed protein modeling analysis of variations found in Sanger sequencing using the Swiss-Model Online software. We used the genomic analysis software Serial Cloner v.2.6 to manually evaluate the mutations identified comparing it to Ensembl GRCh37 assembly version of the *ENG* gene.

Results

We found a single nucleotide deletion c.408delA in exon 4 of the *ENG* gene that has not been previously reported (Figure 2). The deletion causes a frameshift in exon 4, generating an early termination of the protein at aminoacid position 162 instead of the normal length protein of 658 aa. This truncated protein lacks the main functional endoglin sites like residues 270-282 that are essential for junction with BMP9, a major component in the TGF-ß signaling pathway; and the cell attachment site at residues 399-401 (Figures 3 and 4).

Figure 1: Pedigree of the affected family

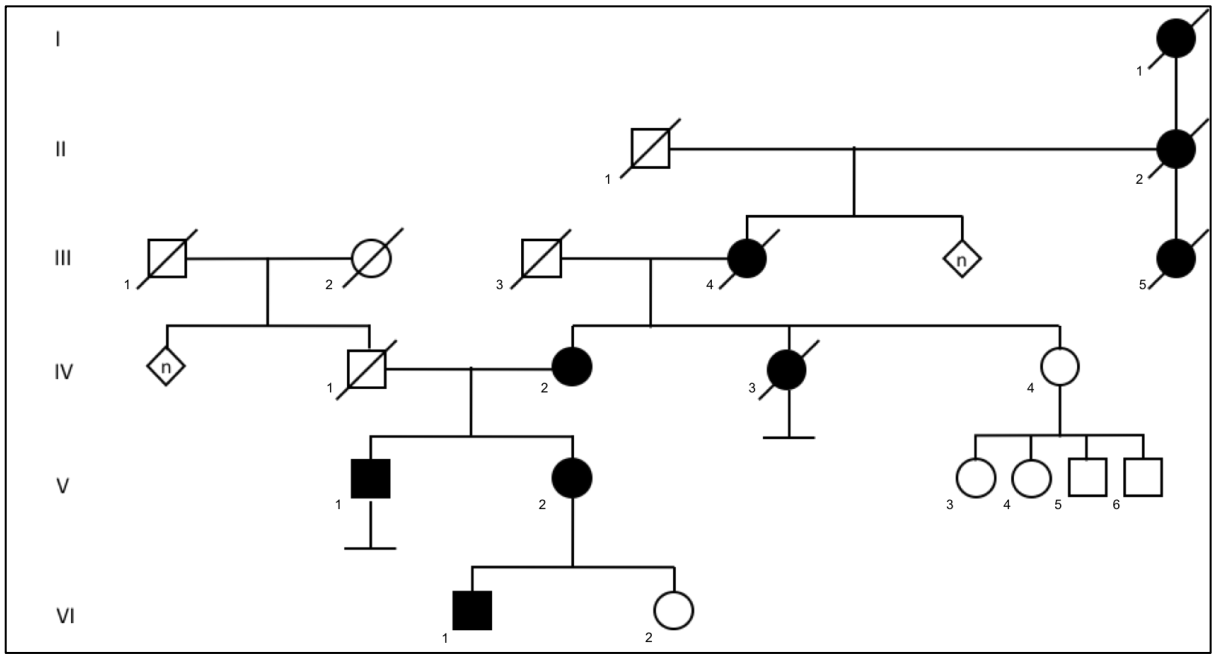

Figure 2: Normal and mutated exon 4 sequences (reverse) of the *ENG* gene.

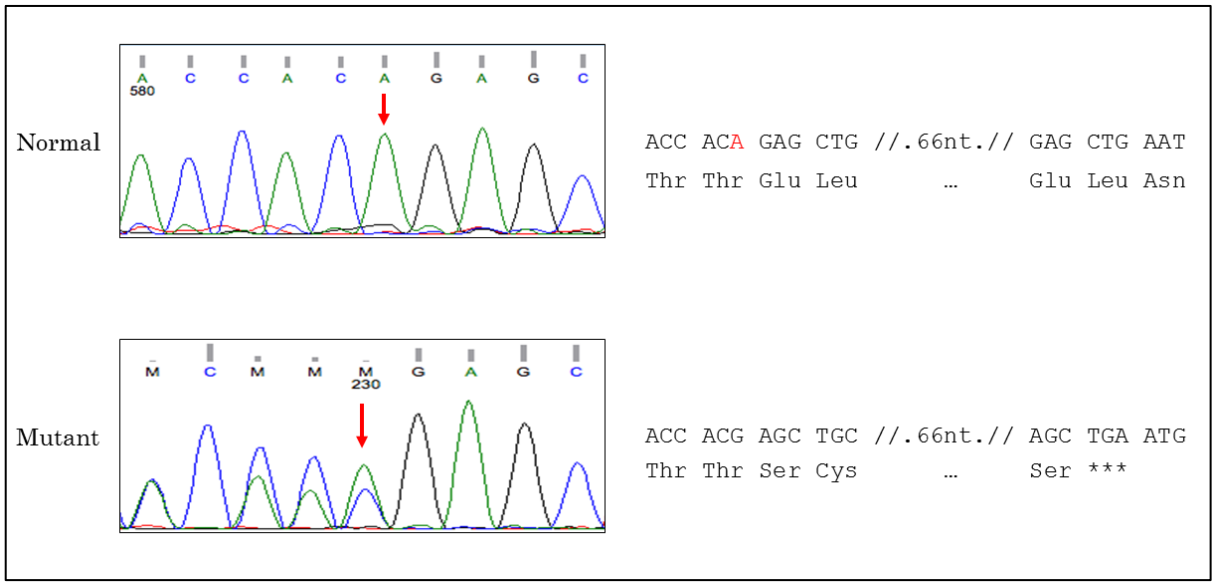

Figure 3: Diagram of the normal and mutated protein. The essential segments for the normal function of the protein are depicted.

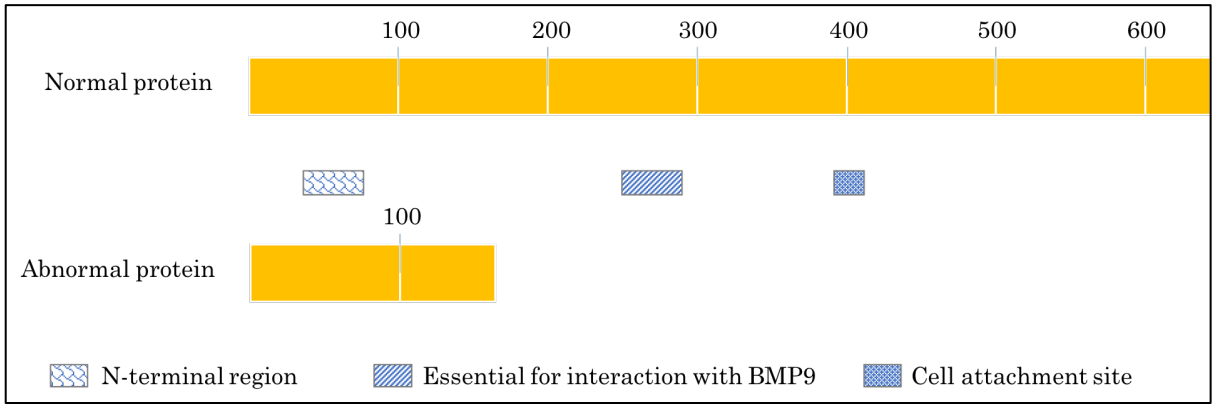

Figure 4: Modeling of the normal endoglin protein (left) and the mutated protein (right).